

REMARKS

Entry of the foregoing and further and favorable consideration of the subject application on the merits are respectfully requested.

As correctly stated in the Official Action, claims 17-33 are pending in the present application. Claims 31 and 33 stand withdrawn from consideration. Claims 17-30 and 32 stand rejected.

By the present amendment, claims 18, 19, and 21-23 have been canceled, without prejudice to or disclaimer of the subject matter contained therein. Claims 17, 20, 24-30, and 32 have been amended. New claims 34-36 have been added. Support for the amendment to claim 17 can be found, at least, in previous claims 23, 29, 1, and 18, and in the specification on page 5, lines 2-11. Support for the amendment to claim 29 can be found, at least, on page 9, lines 26-27. Support for new claims 34-36 can be found, at least in claim 27, and in the specification in the examples and on page 8, lines 6-9 and page 9, lines 26-27. No new matter has been added.

Priority Document

The Office Action Summary indicates that the certified copy of French priority application No. 99/12410 was not received from the International Bureau. Applicants are in the process of procuring a certified copy of this priority document and will submit it as soon as available.

Information Disclosure Statement

The Office Action indicates that WO 99/40203 has not been considered as no English translation was provided. Applicants submit herewith U.S. Patent No. 6,218,139 which corresponds to the U.S. national phase application of WO 99/40203.

Claim Objections

Claims 18 and 24 stand objected to due to the recitation of the APAT abbreviation. Claim 18 has been canceled by the present amendment, thereby mooting this objection as it applies to this claim. Without conceding to the merits of this objection, and solely in an effort to expedite prosecution, claim 24 has been amended to recite the meaning of the APAT abbreviation as suggested by the Examiner on page 4 of the Office Action. Withdrawal of this objection is respectfully requested.

Claims 21, 25, and 30 stand objected to as containing typographical or grammatical errors. Claim 21 has been canceled by the present amendment, thereby mooting this objection as it applies to this claim. Without conceding to the merits of this objection, and solely in an effort to expedite prosecution, by the present amendment, claims 25 and 30 have been amended as suggested by the Examiner on page 4 of the Office Action. Withdrawal of these objections is respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 17-30 and 32 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 18, 19, and 21-23 have been canceled by the present amendment, thereby mooted this rejection as it applies to these claims.

Claims 17, 30, and 32 are allegedly indefinite due to the recitation of *Cyp7b*. Without conceding to the merits of this rejection and solely to expedite prosecution, independent claims 17 and 32 now recite that the yeast cells are transformed with the rat *Cyp7b* gene. Additionally, claims 17 and 32 also recite that the transformation results in the expression of the 7 α hydroxylase enzymatic activity encoded by the rat *Cyp7b* gene which catalyzes 7 α hydroxylation of pregnenolone and DHEA. Withdrawal of this rejection is respectfully requested.

Claim 20 is allegedly indefinite due to the recitation of “precursor contains a 7 position.” Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claim 20 has been amended to recite that the “precursor can be hydroxylated at position 7” as suggested on page 5 of the Office Action. Withdrawal of this rejection is respectfully requested.

Claim 24 is allegedly indefinite due to the recitation of “*atf2* gene.” Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claim 24 has been amended to recite “the *atf2* gene encoding said acetyl coenzyme A-pregnenolone acetyltransferase activity.” Withdrawal of this rejection is respectfully requested.

Claims 24 and 28 are allegedly indefinite due to the recitation of “activity of said yeasts has been rendered low.” Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claims 24 and 28 have been

amended to recite a lack of APAT activity resulting from inactivation of the *atf2* gene or use of an *atf2*- mutant (claim 24) or that the yeast cells lack 17- β -hydroxysteroid dehydrogenase activity when compared to wild type yeast cells (claim 28).

Withdrawal of this rejection is respectfully requested.

Claim 26 is allegedly indefinite due to the recitation of "wherein said dehydrogenase activity is a 17-dehydrogenase activity which produces a 17-hydroxylated derivative." Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claim 26 has been amended to recite that "the dehydroxygenase activity is a 17 β -hydroxysteroid dehydrogenase activity which catalyzes the production of a steroid precursor at position 17." Withdrawal of this rejection is respectfully requested.

Claim 27 is allegedly indefinite due to the recitation of "wherein said dehydrogenase activity is carried by the *yil124w* gene." Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claim 27 has been amended to recite "wherein said 17- β -hydroxysteroid dehydrogenase activity is that encoded by the *yil124w* gene." Withdrawal of this rejection is respectfully requested.

Claim 28 is allegedly indefinite due to the recitation of "method of claim 17 wherein the 17-dehydrogenase activity." Initially, Applicants note that the claim does not refer to the method of claim 27. Rather, claim 28 specifies a new embodiment of the claimed process (inactivation of the *yil124w*-encoded enzymatic activity), which is unrelated to claim 27. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, claim 28 has been amended to recite that the "yeast cells further lack a 17 β -hydroxysteroid dehydrogenase activity when

compared to wild type yeast cells.” Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 17-30 and 32 stand rejected under 35 U.S.C. § 112, first paragraph, as lacking written description. The Office Action asserts that the specification only discloses a few species of genes, steroid precursors, yeast strains, and modifications and therefore allegedly lacks written description for the full scope of the claims. Claims 17-30 and 32 also stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. The Office Action asserts that the specification is not enabling for the full scope of the claims.

Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, the current claims are directed to a method for producing hydroxylated and/or acetylated steroids from DHEA or pregnenolone using *Saccharomyces* yeast cells deficient in APAT activity and producing the rat *Cyp7b* gene product and the modified yeast strains themselves.

In this regard, Applicants draw the Examiner's attention to Example 8 which clearly demonstrates that *S. cerevisiae* yeast strains deficient in APAT activity and transformed by the rat *Cyp7b* gene are suitable to practice the claimed process (e.g., the TGY206p-pTg 14014 strain). The TGY206-pTg14014 strain comes from the parental strain TGY186 (in which the *atf2* gene has been inactivated by insertion of an expression block, resulting in the loss of *atf2*-encoded APAT activity (see specification at p. 14, ll. 7-15) and was transformed by the pTG14014 plasmid which incorporates the *Cyp7b* gene under the control of TEF-1 promoter. As discussed at

the top of page 20 of the specification, TGY206p-pTg14014 strain is capable of providing complete bioconversion from DHEA, allowing recovery of almost 100% of 7 α hydroxylated DHEA from the culture medium.

Applicants further emphasize that *Saccharomyces* strains deficient in APAT activity can be readily generated by the skilled artisan without undue experimentation. A number of routine techniques exist in the art for inactivating expression of a given gene in a yeast genome, especially when the gene sequence is well known in the art. The present application illustrates inactivation of APAT activity from a *S. cerevisiae* genome by insertion of an exogenous expression cassette into the APAT-encoding sequence (*aff2* locus). However, other techniques can also be suitably practiced such as the introduction of mutation or deletion within the APAT coding sequences and knock-out techniques. Applicants respectfully submit that the nature of the modifications performed to suppress expression of the yeast APAT activity or to inactivate the corresponding gene are irrelevant to the presently claimed process. All of these techniques lead to a similar phenotype, *i.e.*, lack of APAT activity. Moreover, the specification provides guidance as to how to generate APAT-deficient yeast strains. See, *e.g.*, specification, p. 7, ll. 12-21.

The present claims recite a method for producing hydroxylated and/or acetylated steroids from the steroid precursors DHEA and pregnenolone. Applicants respectfully submit that the hydroxylated and/or acetylated steroid products that can be obtained from these precursors following enzymatic bioconversion by the 7 α hydroxylase encoded by the rat *Cyp7b* gene are thus predictable by one skilled in the art.

In light of the above, Applicants respectfully submit that the presently claimed invention fully complies with both the written description and enablement requirements of 35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusions

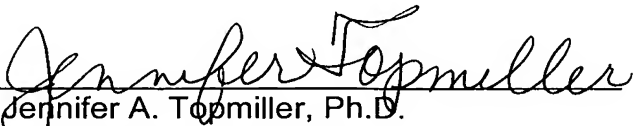
From the foregoing, further and favorable consideration of the subject application on the merits in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

If there are any questions concerning this amendment, or the application in general, the Examiner is respectfully requested to telephone Applicant's undersigned representative so that prosecution may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: April 26, 2004

By: 
Jennifer A. Topmiller, Ph.D.
Registration No. 50,435

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620